Thrombin Promotes Intervertebral Disc Degeneration via Macrophage M1 Polarization and Angiogenesis in a Mouse Tail Puncture Model

Rikito Tatsuno[1], Takashi Ando[1,2], Taro Fujimaki[1], Yoshihiro Takayama[3], Tetsuro Ohba[1], Hirotaka Haro[1]
[1] University of Yamanashi, Yamanashi, Japan [2] Koshu Rehabilitation Hospital, Yamanashi, Japan [3] Yamanashi Kosei Hospital, Yamanashi, Japan
Email: rtatsuno@yamanashi.ac.jp

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INTRODUCTION: Low back pain affects individuals across all age groups, from adolescents to the older adults. It is associated with frailty and sarcopenia in older adults, leading to impaired mobility and poor overall health. Owing to global aging demographics, the number of individuals affected by low back pain is expected to increase significantly. Physiologically, the intervertebral disc (IVD) is an avascular structure composed primarily of cartilage matrix. IVD degeneration triggers inflammation and ingrowth of sensory nerves, contributing to pain development. Thrombin, a coagulation factor, is known to promote IVD degeneration by regulating the production of monocyte chemotactic protein-1 (MCP-1) and matrix metalloproteinase-3 via the MAPK-ERK and PI3/AKT pathways. Macrophages are broadly classified into the pro-inflammatory M1 and anti-inflammatory M2 subtypes. In the context of IVD degeneration, M1 macrophages dominate during the early phase, whereas M2 macrophages increase in later stages. However, the precise role of M1/M2 polarization in IVD inflammation remains poorly understood. This study aimed to investigate whether thrombin induces macrophage polarization and contributes to IVD degeneration.

METHODS: Bone marrow-derived macrophages were isolated from mouse femurs, cultured in Dulbecco's modified Eagle's medium (DMEM), and stimulated with thrombin (100 nM). For *ex vivo* organ culture, mouse intervertebral discs (mIVDs) were harvested and cultured in DMEM with or without thrombin (100 nM; Prolytix) or the protease-activated receptor 1 (PAR1) inhibitor SCH79797 (Santa Cruz Biotechnology). Homozygous wild-type male C57BL/6J mice (5–6 weeks old; CLEA Japan) were assigned to puncture or control groups (n = 3 per group). Caudal IVD degeneration was induced by puncturing the coccygeal discs 8–9 with a 27G needle. SCH79797 (4μg/kg) was administered intravenously twice weekly for 3 weeks. Total RNA was extracted from mIVDs for qRT-PCR analysis of MCP-1, Cluster of differentiation 86 (CD86), CD163, and vascular endothelial growth factor (VEGF; Applied Biosystems). Western blotting was performed to detect inducible nitric oxide synthase (iNOS; R&D Systems), arginase-1 (Cell Signaling Technology), CD86 (Novus), and CD163 (Abcam, Cambridge, UK). Histological analysis included Safranin-O and hematoxylin and eosin staining. Immunohistochemistry was performed to evaluate the levels of thrombin (Santa Cruz Biotechnology), MCP-1 (Cell Signaling Technology), Iba-1 (Fujifilm), iNOS (R&D Systems), arginase-1 (Cell Signaling Technology), CD86 (Novus), CD163 (Abcam), VEGF (Santa Cruz Biotechnology), and CD31 (Cell Signaling Technology). Statistical significance was set at $p < 0.05$.

RESULTS: *In vitro*, stimulation of bone marrow-derived macrophages with thrombin enhanced the expression of M1 markers (CD86 and iNOS) and reduced the expression of M2 markers (CD163 and arginase-1), as confirmed by qRT-PCR and western blotting, indicating M1 polarization (Fig. 1). In an *ex vivo* model, thrombin treatment caused a loss of Safranin-O staining and significantly increased MCP-1 mRNA expression. Thrombin also increased VEGF mRNA levels, whereas SCH79797 suppressed this increase. Caudal disc puncture induced IVD degeneration and increased thrombin expression *in vivo,* particularly in the nucleus pulposus and cartilage endplates. The punctured discs exhibited increased CD86 and iNOS expression, with persistently low CD163 levels. Administration of SCH79797 suppressed the puncture-induced increase in M1 macrophage polarization (Fig. 2). VEGF-positive cells were more abundant in the punctured discs than in controls. These findings suggested that thrombin promotes M1 macrophage polarization, VEGF production, and angiogenesis in degenerated IVDs via PAR1 signaling.

DISCUSSION: This study demonstrated that thrombin induces M1 macrophage polarization through PAR1 signaling and accelerates disc degeneration by promoting VEGF-mediated angiogenesis. The novelty of this study lies in its immunological perspective, which highlights the role of thrombin in modulating macrophage polarization during IVD degeneration. M1 macrophages are known to secrete proinflammatory cytokines and contribute to tissue destruction. Our data confirm that thrombin stimulation drives M1 polarization in bone marrow-derived macrophages. Additionally, VEGF production was enhanced, suggesting that angiogenesis was induced within the IVD. Although the IVD is normally avascular, previous studies have reported neovascularization and nerve ingrowth during degeneration, which are thought to contribute to pain. Our findings support this hypothesis. Furthermore, the administration of the PAR1 inhibitor SCH79797 significantly attenuated disc degeneration. PAR1 inhibitors such as vorapaxar are already in clinical use for treating cardiovascular conditions and have well-established safety profiles. Therefore, repurposing such agents for degenerative disc disease could expedite clinical translation and therapeutic implementation. Overall, these findings suggested a novel therapeutic strategy for chronic low back pain by targeting immune dysregulation during disc degeneration.

SIGNIFICANCE: This study identifies thrombin as a key promoter of intervertebl disc degeneration via macrophage polarization toward the M1 phenotype and VEGF-mediated angiogenesis. Targeting PAR1 signaling may provide a promising and clinically translatable strategy for the treatment of degenerative disc diseases and chronic low back pain.

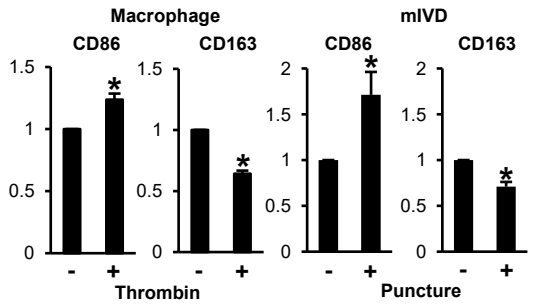

Fig. 1 Thrombin stimulation of macrophages and puncture of mouse intervertebral discs promote polarization toward the M1 macrophages. CD; Cluster of differentiation, mIVD; mouse intervertebral disc

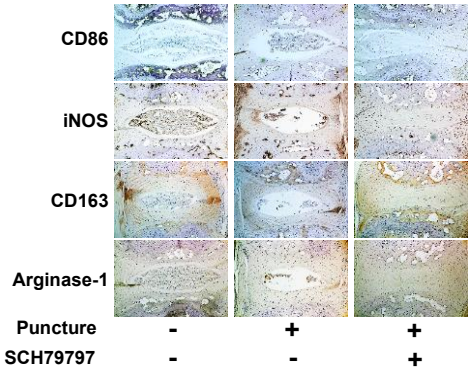

Fig. 2 PAR1 antagonist (SCH79797) inhibits M1 polarization of macrophages in a mouse intervertebral disc puncture model. Immunohistochemistry was performed using antibodies against CD86, iNOS, CD163, and arginase-1. CD; Cluster of differentiation, iNOS; inducible nitric oxide synthase, PAR1; protease-activated receptor 1